# Distribution of Tobacco Mosaic Virus in Etiolated Tobacco Leaf Cells Infected with two Viruses

Y. Honda and C. Matsui

Plant Pathology Laboratory, Faculty of Agriculture, Nagoya University, Nagoya, Japan.
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ABSTRACT

Etiolated leaf cells of *Nicotiana tabacum* were studied by electron microscopy after inoculation with cucumber mosaic virus and tobacco mosaic virus (TMV). In addition to TMV particles within the cytoplasm, large and small amounts of rod-shaped particles were observed within the matrix of the nuclei and the proplastids, respectively. The rod-shaped particles within the nuclei were oriented at random, whereas the particles within the proplastids were arranged parallel to each other. Since their diameters, as well as similar particles in the cytoplasm, were about 8-9 nm, they were believed to be TMV particles. They were probably formed within the nucleus and proplastid, respectively. The rod-shaped particles could not be detected within the nuclei and plastids of TMV-infected leaf cells grown in daylight. Phytopathology 61:759-762.

The intracellular sites of virus formation have been discussed by several reviewers (1, 5, 15, 18). Most previous electron microscope studies revealed that intracellular virus particles were limited to the cytoplasm. Recent electron microscopy showed tobacco mosaic virus (TMV) particles within the nuclei and plastids (6, 9, 10, 16, 20). These findings provide a morphological basis for several approaches to research on the functional significance of the nuclei and plastids in TMV formation. In a previous paper (10), the authors reported that a small number of TMV particles occurred in the nuclei of green tobacco leaves infected with both TMV and cucumber mosaic virus (CMV), but there were no TMV particles in the nuclei of hosts that were infected only with TMV. The present paper describes more evidence of TMV particles within the nuclei and plastids of etiolated leaves, and supports our previous report (10).

MATERIALS AND METHODS.—Seedlings of *Nicotiana tabacum* L. 'Bright Yellow' were grown in a greenhouse at 25 C. Upper young leaves were wrapped with black polyethylene covers, and one-half of each of three lower leaves was inoculated mechanically with the ordinary strain of CMV. After 7 days, the opposite halves were inoculated mechanically with the ordinary strain of TMV. Thirteen days later, the covers were removed and etiolated young leaves were examined (Fig. 1).

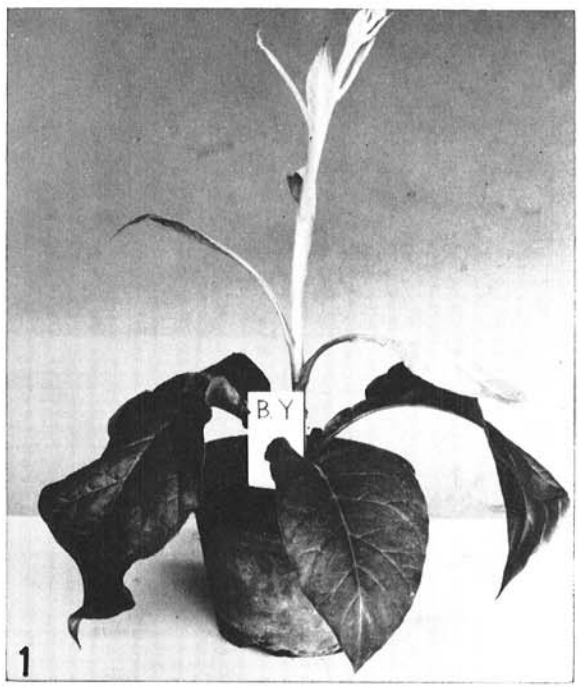
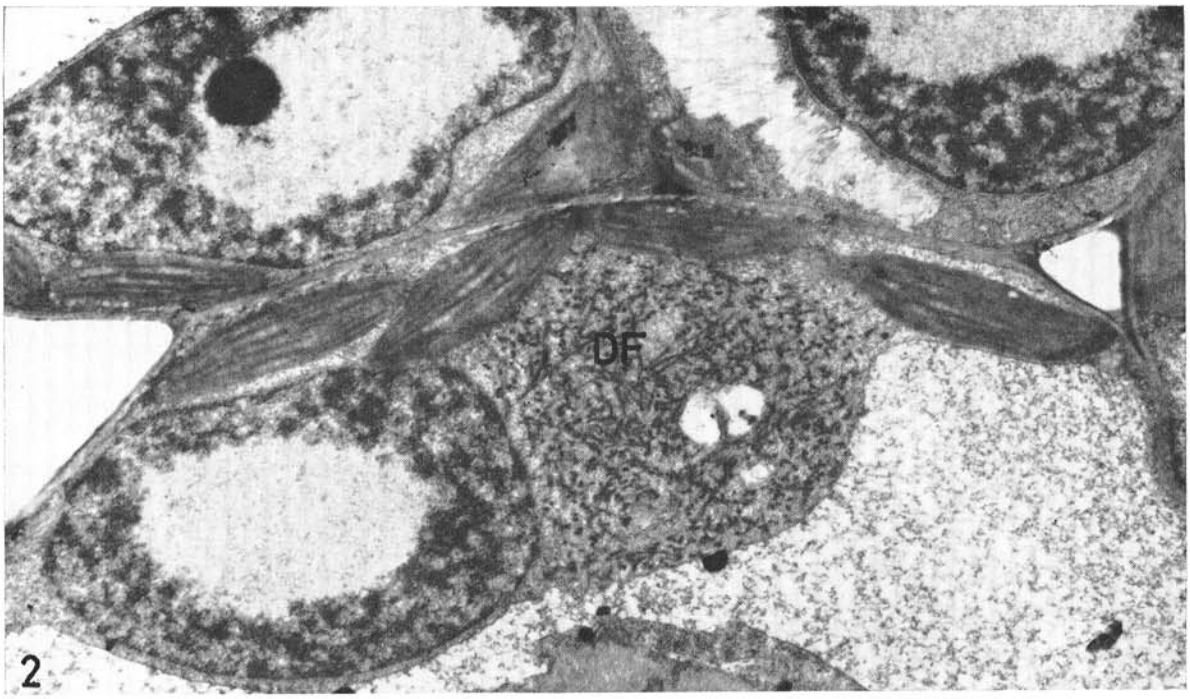



**Fig. 1.** Etiolated tobacco plant infected with cucumber mosaic virus and tobacco mosaic virus.

Pieces of the etiolated young leaves were fixed with 4% formaldehyde and 5% glutaraldehyde at 5 C for 1 hr, and washed with Milloning's phosphate buffer (pH 7.5). They were postfixed with 2% osmium tetroxide in Milloning's phosphate buffer (pH 7.5) at 5 C for 5 hr. After washing and dehydration, they were embedded in a mixture of Dow epoxy resin 736 and Epon 812 (12). Thin sections stained with uranyl acetate and lead citrate were examined in a JEM-T7S electron microscope (JEOL Co., Ltd.). For comparison, healthy or TMV-inoculated tobacco plants whose upper leaves were wrapped with black polyethylene covers were examined.

RESULTS AND DISCUSSION.—*Tobacco mosaic virus in nuclei.*—In etiolated leaf cells infected with CMV and TMV, large electron-transparent areas were observed within the nuclear matrix (Fig. 2). No membranes were around them. The nucleoli were often surrounded by electron-transparent areas (Fig. 3), but these areas were never encountered within the nucleoli. Nuclei with electron-transparent areas were encountered in mesophyll cells, epidermal cells, and trichome cells. About 600 different nuclei were examined in an electron microscope. About 90 nuclei contained electron-transparent areas, and the cells with such nuclei tended to occur together in groups (Fig. 2). At higher magnification, these electron-transparent areas were seen to be filled with rod-shaped particles whose diam was about 8-9 nm, and the nuclear matrix was scarce within the areas (Fig. 3, 4). It is likely that these rod-shaped particles in the nuclei correspond to TMV, because their average diam (8-9 nm) was in good agreement with that of the RNA helix in purified TMV particles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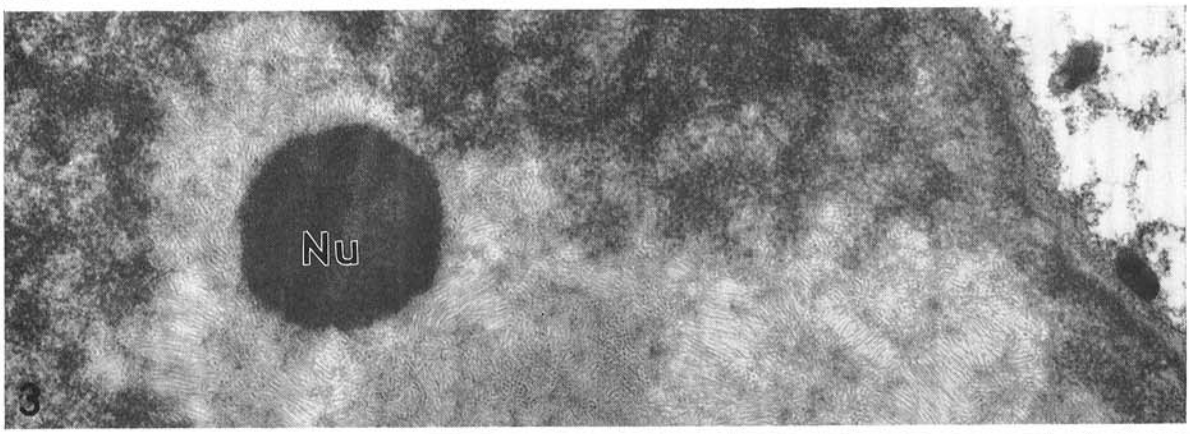
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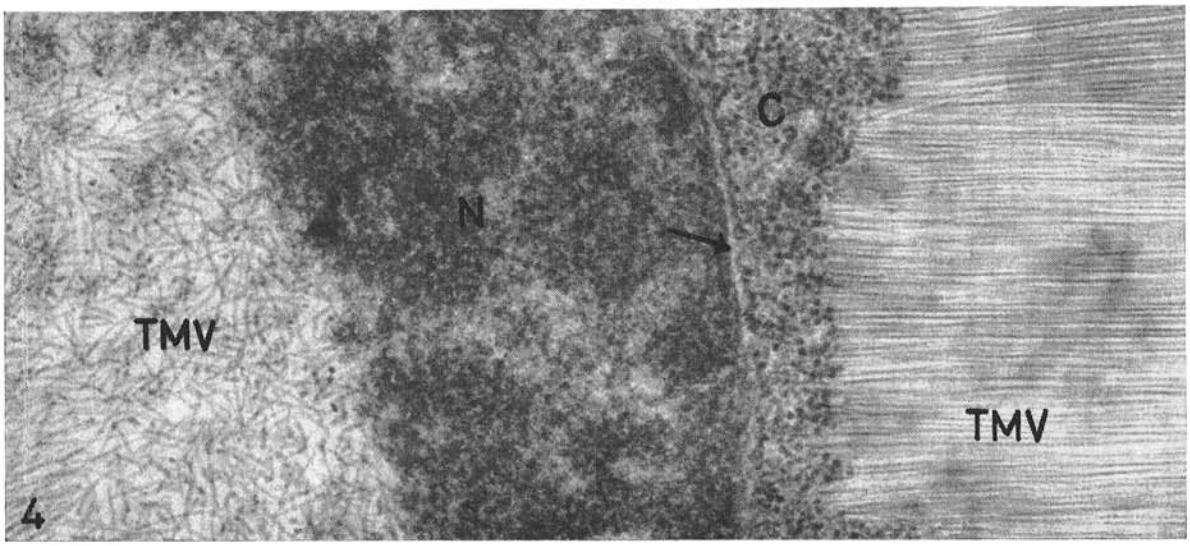
C
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**Fig. 2-4.** Intranuclear tobacco mosaic virus (TMV) particles in etiolated tobacco leaf cells infected with cucumber mosaic virus (CMV) and TMV. **2)** Three large electron-transparent areas within the nuclear matrix. In the cytoplasm, dense filaments (DF) are observed near the nucleus. (×9,000) **3)** Nucleolus (Nu) surrounded by electron-transparent areas which were filled with rod-shaped particles. Nucleolus is intact and TMV particles are never seen within it. (×23,000) **4)** TMV particles within the nucleus (N) and cytoplasm (C). Although cytoplasmic TMV particles are in parallel arrangement, intranuclear TMV particles are arranged irregularly. The nuclear membrane is intact as indicated by arrow. (×51,000)

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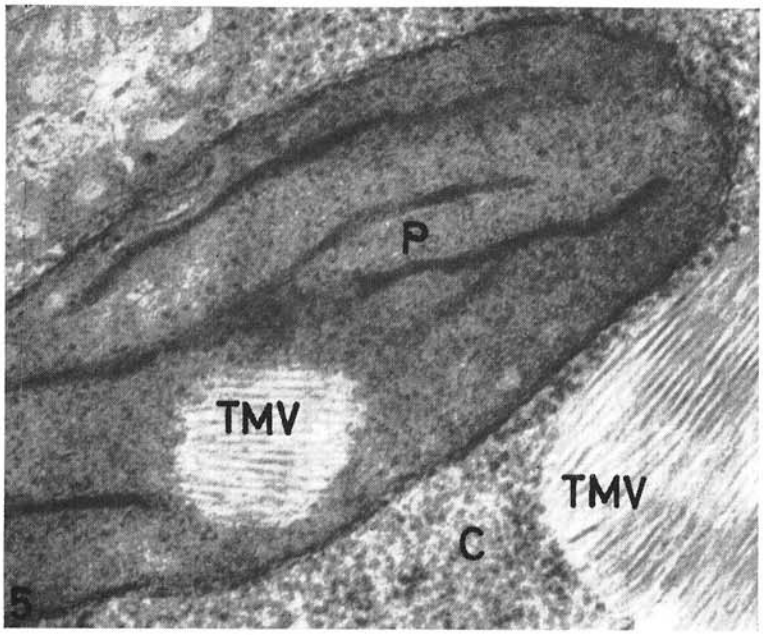


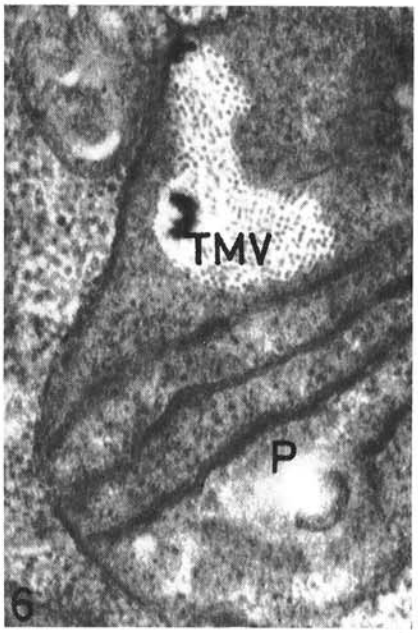


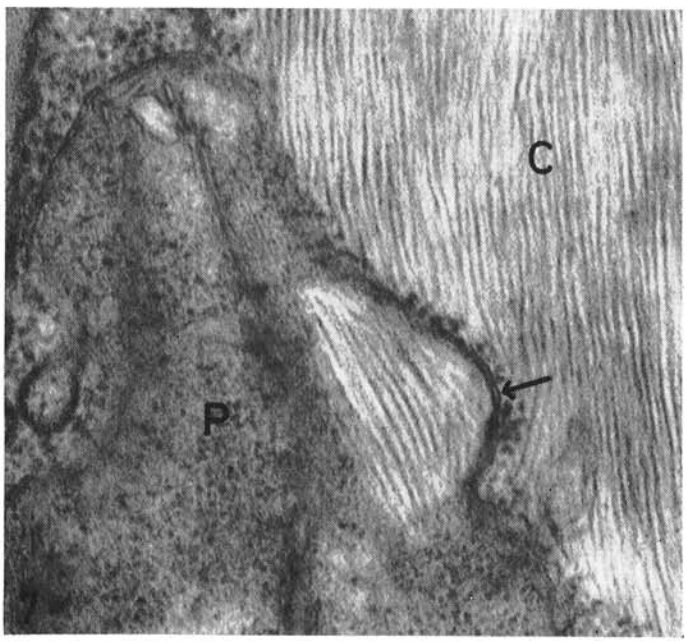


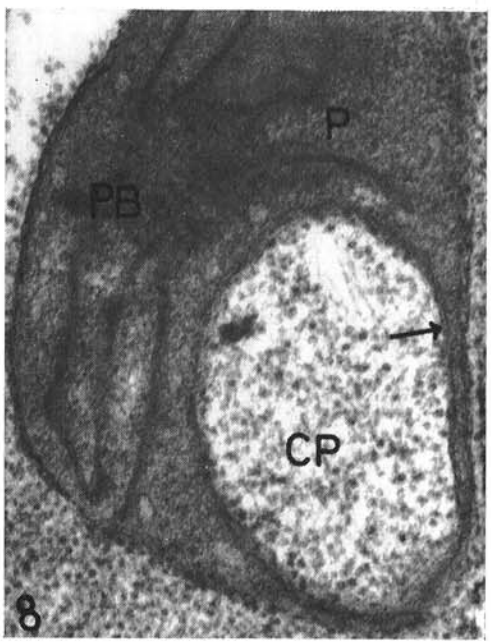


**Fig. 5-8.** Tobacco mosaic virus (TMV) particles within the proplastids of etiolated leaf cells infected with cucumber mosaic virus and TMV. **5)** Longitudinal section of TMV particles within the proplastid (P) and cytoplasm (C). Arrangement of TMV particles within the proplastid and cytoplasm is nearly parallel in each case. (×35,000) **6)** Transverse section of TMV particles within the proplastid (P). The mass of TMV particles has no membranes around it. (×34,000) **7)** TMV particles within the proplastid (P) and cytoplasm (C). The limiting membrane of the proplastid is intact as indicated by arrow. (×56,000) **8)** TMV particles within the cytoplasmic protrusion (CP) surrounded by a limiting membrane (arrow) of the proplastid (P). Note the prolamellar body (PB). (×34,000)

(8 nm) (2, 7). The arrangement of intranuclear TMV particles was irregular (Fig. 3, 4), though cytoplasmic TMV particles were in parellel arrangement (Fig. 4). Although the dense filaments described first by Shalla (19) were observed near the nuclei (Fig. 2), these filaments were not in the nuclei. The limiting membranes of the nuclei with TMV particles were intact (Fig. 4). No nuclei with TMV particles were detected in the etiolated leaf cells infected only with TMV.

There are several reports concerning virus particles, other than TMV, within the nuclei (3, 4, 8, 11, 13, 14, 17, 21, 22), and most intranuclear virus particles were considered to be formed in situ. In the present study, we found no evidence to disprove our previous assumption that the nucleus has the ability to form TMV, but this ability is not expressed under experimental conditions used commonly in TMV infection (10).

*Tobacco mosaic virus in proplastids.*—In the proplastids of etiolated leaf cells doubly infected with CMV and TMV, small electron-transparent areas were encountered. At higher magnifications, rod-shaped particles were frequently observed in these areas (Fig. 5). Presumably, these rod-shaped particles correspond to TMV particles, because their diam and arrangement observed in longitudinal and transverse sections (Fig. 5, 6) were similar to those of TMV particles in the cytoplasm. There were no membranes around the electron-transparent areas, and the matrix of proplastids was scarce within them. The limiting membranes of proplastids having electron-transparent areas were intact (Fig. 7). In the present study, about 1,500 proplastids were examined; about 120 of them contained TMV particles. The actual number of proplastids containing TMV particles may be greater, because the masses of TMV particles in the proplastids were so small that they were apt to be overlooked. The number of proplastids containing TMV particles in the etiolated leaf cells infected with TMV was smaller than that of the etiolated leaf cells doubly infected with CMV and TMV. In addition to the electron-transparent areas with TMV particles, cytoplasmic protrusions containing ribosomes were observed in the proplastids. Some protrusions contained cytoplasmic TMV particles (Fig. 8).

In contrast to the relatively abundant information on intranuclear virus particles, information concerning virus particles within plastids is scarce (6, 9, 16, 20). In general, it is considered that the particles within plastids are formed in situ as are the intranuclear virus particles. The general view of TMV particles within plastids in the present study was similar to those reported previously (6, 9, 16, 20). The present observations offer no evidence refuting the conclusion that virus particles do occur within plastids.

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